Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 4288–4292, May 1994
Neurobiology

Evidence for a peripheral olfactory memory in imprinted salmon

(patch clamp/olfactory receptor cells/homing/chemoreception/learning)

GABRIELLE A. NEVITT*†, ANDREW H. DITTMAN‡§, THOMAS P. QUINN‡, AND WILLIAM J. MOODY, JR.*

*Department of Zoology, NJ-15, ‡School of Fisheries, WH-10, and §Department of Pharmacology, SJ-30, University of Washington, Seattle, WA 98195

Communicated by John E. Halver, January 12, 1994

ABSTRACT **The remarkable homing ability of salmon relies on olfactory cues, but its cellular basis is unknown. To test the role of peripheral olfactory receptors in odorant memory retention, we imprinted coho salmon (*Oncorhynchus kisutch*) to micromolar concentrations of phenyl ethyl alcohol during parr–smolt transformation. The following year, we measured phenyl ethyl alcohol responses in the peripheral receptor cells using patch clamp. Cells from imprinted fish showed increased sensitivity to phenyl ethyl alcohol compared either to cells from naive fish or to sensitivity to another behaviorally important odorant (L-serine). Field experiments verified an increased behavioral preference for phenyl ethyl alcohol by imprinted salmon as adults. Thus, some component of the imprinted olfactory homestream memory appears to be retained peripherally.**

Salmon are well known for their ability to return to the stream in which they were spawned to reproduce and complete their life cycle. The sensory basis for home stream selection is olfactory: during a sensitive developmental period (parr–smolt transformation), salmon imprint to site-specific odors, and adults later use this odorant memory as a migratory cue to guide them back to their natal streams (1, 2). In support of this hypothesis, numerous investigations have shown that salmon can detect, imprint, and home to synthetic chemical odorants such as morpholine or phenyl ethyl alcohol (PEA) at submicromolar concentrations (3–5).

Despite the extensive behavioral evidence linking olfactory imprinting to salmon homing, attempts to trace the neurobiological basis of homestream memory and recognition have proven inconclusive. Studies using electroolfactogram recordings have reported that imprinted fish show significantly greater responses to specific imprinted odorants than do nonimprinted fish (6, 7), but other researchers have claimed that these "imprinted" odorants consistently fail to elicit increased electrical activity at the level of the olfactory bulb in imprinted as compared to nonimprinted fish (8–10).

In this study, we have combined patch clamp recording and behavioral imprinting assays to test directly the hypothesis that a change in the sensitivity of peripheral olfactory receptor cells contributes to establishing an olfactory memory in salmon. Our results demonstrate that plasticity in the peripheral olfactory system at the receptor cell level is linked to olfactory imprinting (11, 12).

METHODS

All investigations were performed on coho salmon spawned from the University of Washington's School of Fisheries stock. Details of imprinting paradigms, electrophysiological techniques, and behavioral methods are given below.

Imprinting Procedures. *Determination of parr–smolt transformation.* To achieve parr–smolt transformation by the first spring (0-age), fish were maintained at slightly elevated water temperatures (14–16°C) and fed ad libitum. Fish were hand-culled to avoid exposing them to harsh drugs or other chemicals commonly used as disease preventatives in salmon culture. Parr–smolt transformation was determined by coloration changes, a behavioral tendency to orient downstream, and the ability of fish to maintain blood plasma Na^+ levels to within 5% during a 48-hr exposure to salt water (freshwater exposure: 152.9 ± 1.2 meq of Na^+ per liter of blood plasma, $n = 10$; saltwater exposure: 159.0 ± 1.7 meq of Na^+ per liter of blood plasma, $n = 8$) (13–15). To determine plasma Na^+ levels, salmon were transferred from their rearing hatchery (13°C) to holding tanks containing either aerated fresh filtered hatchery or sea water (27 ppt; 12°C). After 48 hr, blood was collected using heparinized capillary tubes and immediately centrifuged to remove plasma. Plasma osmolality (mosM) was determined in duplicate using a vapor pressure osmometer and converted to plasma Na^+ (meq/liter) using an empirically derived regression equation for coho salmon: (meq/liter = 0.391 × mosM + 30.708, $R^2 = 0.87$, $P < 0.01$) (16).

Imprinting paradigm. Once parr–smolt transformation was established for the cohort, we separated fish into experimental and control groups. The experimental group was imprinted for 10 days by adding phenyl ethyl alcohol (PEA) to the water intake to reach an effective overall concentration of 10^{-7} M; control fish were never exposed to PEA. Experimental (PEA-exposed) and control (PEA-naive) groups were marked and transferred to a common outdoor freshwater rearing facility.

Electrophysiology. *Recording techniques.* Patch pipets were prepared from standard VWR micropipet glass using a two-stage pull and were not fire-polished. Pipets were filled with salmon Ringer solution [composition (mM): 130 NaCl, 3 $CaCl_2$, 1 $MgCl_2$, 5 KCl, 5.5 glucose, and 10 Hepes buffered to pH 7.4]. Pipet resistances were 5–10 MΩ. Voltage outputs were recorded and referenced to a silver wire isolated from the bath via an agar bridge. Records were filtered at 2 kHz using a filter with an eight-pole Bessel characteristic. Data were digitized, stored, and processed using a standard laboratory microcomputer.

Experimental protocol. Olfactory receptor cells were removed and isolated for cell-attached patch clamp recording using the techniques of Nevitt and Moody (17). Upon the formation of a 5 to 20 GΩ seal, the pipet potential was held slightly negative, and spontaneous activity was recorded. The odorant pipet was lowered into the chamber and positioned near the cell such that both the odorant pipet and the cell were situated near the bottom of the chamber, close to the chamber floor. A brief (i.e., 0.5 sec) spritz of stimulus odorant (either PEA or L-serine; Figs. 1 and 2, arrows) was then delivered at pipet concentrations of 10^{-5}–10^{-8} M from

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: PEA, phenyl ethyl alcohol.

†To whom reprint requests should be addressed at the present address: Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

a distance within 100 μm of the dendritic knob of the cell. The odorant thus had to diffuse this distance before reaching the cell. We specifically devised this stimulus paradigm to eliminate or greatly reduce mechanical artifacts caused by spritzing. Based on measurements of the initial dispersal of experimental dye spritzes, and assuming a diffusion coefficient of 10^{-6} cm^2/sec for each odorant, we estimate that the actual odorant concentration reaching cells was on the order of a 1000-fold less than the pipet application concentration, with peak concentration reaching cells within 20 sec (18). Spritz pulse, pipet diameter, and pipet–cell distance were kept constant to ensure control between trials. Each cell was stimulated using only one pipet concentration.

Analysis. Data were collected and digitized blindly and analyzed for gross changes in electrical activity coincident with odorant application. Negative deflections suggesting inward current activity (19) or biphasic action potentials (20) (e.g., Fig. 1*A*, first trace) were quantified by sampling the first 20 sec (four 5-sec intervals) of activity during a 30- to 40-sec period before the odorant pipet was lowered into the bath. Sample records of this baseline activity are shown in Figs. 1 and 2. An average current value for background activity was subsequently determined by integrating the net inward current for each interval, adding them up and dividing by 4. Average current values for odorant responses were also integrated and averaged over 20 sec (four continuous 5-second intervals), starting at the response onset. We defined the response onset to be the first 5-sec interval showing a 100% increase in background activity within 1 min following odorant application. For cells that did not show such an increase in activity, sampling was initiated at the average time required for the onset of responses in other cells (27 sec). Spiking activity was determined by counting spikes using the same basic procedure, except that spiking events were additionally verified by visual inspection of data records.

Behavior. *Experimental apparatus.* Behavioral experiments were conducted from Nov. 11 to Dec. 12, 1989, in a two-choice arena constructed in a controlled flow experimental stream channel located at the University of Washington's Big Beef Creek Research Station. The experimental arena consisted of two contiguous gravel-lined sections: a large upstream section (23.7 m × 6.2 m, 0.35 m water depth) separated by a low (0.2 m) waterfall from a smaller downstream section (15.3 m × 1.4 m, 0.75 m water depth). The upstream section was divided into two arms by a 10.0-m concrete barrier extending downstream from the channel inlet. Funnel traps constructed in each arm allowed fish to move upstream into either arm but did not allow them to leave. Water for the arena was taken from Big Beef Creek at a point 0.4 km above the channel inlet. During the course of the study, water flow in each arm varied between 50 and 70 liters/sec but was always equivalent in both arms of the maze.

Experimental protocol. On Nov. 11, 1989, 25 tagged mature salmon from each group were transported from Seward Park Hatchery and released into the downstream section of the experimental arena. Fish were allowed to move freely within the arena until they entered a trap. Each day, trapped individuals were removed and their arm choice was recorded. Twice a week, equivalent numbers of new fish from each experimental group were released into the maze to replace them. We continued this procedure until 110 fish from each experimental group had been tested. From Nov. 11 to Nov. 14, 1989, no PEA was metered into the maze and fish making choices during this period were designated as "no PEA present" controls. Between Nov. 15 and Dec. 12, PEA was continuously metered into the inlet of arm B of the maze to give a concentration of 10^{-7} M PEA. The PEA drip was adjusted daily to maintain this concentration throughout the study.

Analysis. The responses of the two experimental groups in the presence and absence of PEA were compared using a χ^2 contingency analysis (21). We assumed that all fish were acting independently. Fish that did not enter either trap were excluded from the analysis.

RESULTS

On December 1, 1987, we collected, pooled, and fertilized 10,000 eggs from adult coho salmon that had returned to spawn at the University of Washington School of Fisheries Hatchery. We reared these fish at the University of Washington until parr–smolt transformation the following spring (22). During the following fall and winter, olfactory receptor cells were isolated from both PEA-exposed and PEA-naive fish using previously described procedures (17). Isolated cells were tested for responsiveness to either PEA or L-serine, a well-established, innate olfactory repellant for salmonids (23, 24). In addition, we performed behavioral experiments on the same cohort to confirm the success of our imprinting paradigm. Details of patch clamp and behavioral results are described below.

Electrophysiology. Olfactory receptor neurons were isolated from fish 6–9 months following our imprinting protocol. To record cellular activity, we used the cell-attached mode of the patch clamp recording method. This approach allowed us to monitor single cell responses to odorants, while avoiding disruption of the intracellular milieu required for odorant transduction and maintenance of whole cell currents (12, 25, 26). Using this recording configuration, cells typically responded to odorants (PEA or L-serine) applied at micromolar pipet concentrations with rapidly activating bursts of inward current or, more variably, biphasic action potentials (see Figs. 1 and 2). The variability in spiking probably reflects subtle differences in the resting potentials of these high-resistance (12) dissociated cells, which we were unable to control using this recording configuration (20). Bursts of inward current were more predictably activated by odorants and were similar to odorant-activated single channel events described elsewhere (19). These bursts reversed direction at positive pipet holding potentials but could not be activated by varying the pipet potential in the absence of odorant or by the application of odorant via the recording pipet. For most recordings, single channel activity was difficult to resolve due to the extremely short (<2 msec) open times of odorant-induced channels.

Qualitatively, olfactory receptor cells isolated from PEA-exposed fish showed strikingly more robust responses to PEA than did cells isolated from PEA-naive fish (Figs. 1 and 3*A*). Quantification of the bursts of inward activity that comprised the bulk of the PEA responses were significantly different between cells isolated from fish with different histories of PEA exposure, but only at higher PEA application concentrations (Fig. 3*A*). This effect was apparently not due to a generalized increase in excitability of odorant responsiveness in these cells, since cells isolated from either PEA-exposed or PEA-naive fish responded similarly to L-serine (Figs. 2 and 3*B*). Although mean averages suggest that, in the absence of PEA exposure during smolting, the receptor cell responsiveness to PEA is reduced (i.e., Fig. 3*A*) (20), the only statement we can make with statistical certainty is that cells isolated from PEA-exposed and PEA-naive fish are different at 10^{-6} M (24). In contrast, differences in responsiveness to L-serine were not significant at any concentration tested (Fig. 3*B*). Neither odorant elicited increases in action potential activity.

Behavior. To confirm the success of our imprinting paradigm, fish of the same cohort were behaviorally tested for responsiveness to PEA in a two-choice, controlled flow experimental stream channel constructed in the field (see

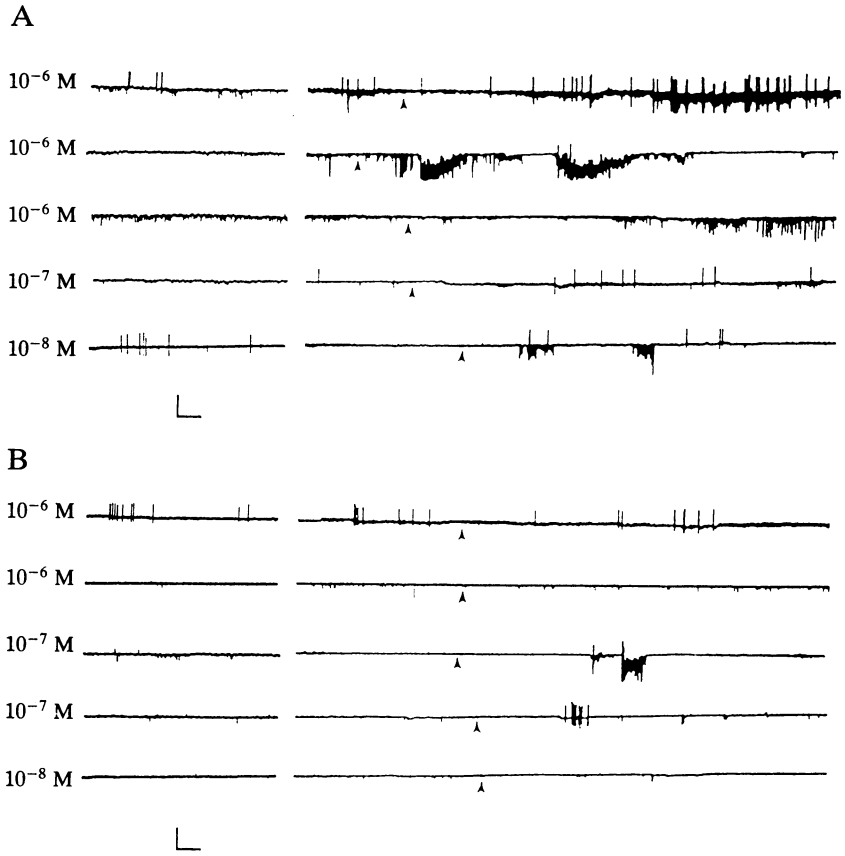

FIG. 1. PEA-induced activity recorded from olfactory receptor cells isolated from experimental (PEA-exposed, *A*) and control (PEA-naive, *B*) fish. Each trace was recorded from a different cell. Odorant-induced bursts of inward current and biphasic action potentials are clearly visible. Arrowheads indicate the time of odorant application. Traces show additional representative baseline activity before the odorant pipet was introduced into the recording chamber. Records containing noise artifacts caused by lowering the odorant pipet into the solution have been removed for clarity. Typical responses are shown for the PEA-exposed group, whereas those shown for the PEA-naive group are the most robust we observed for that group. Experiments were limited to this concentration range because higher pipet concentrations (10^{-5} M) caused cells isolated from PEA-exposed fish to appear to become so leaky that GΩ seals were difficult to maintain. We were therefore unable to statistically examine responses from either group to pipet concentrations higher than 10^{-6} M for this odorant. Note that because PEA was applied from a distance (100 μm), we estimate that the actual odorant concentration reaching the cell to be lower than the pipet application concentration, with peak concentrations reaching cells within 20 sec (see *Methods*) (18). Calibrations: 8 pA; 4 sec; for PEA-exposed, trace 3: 8 pA; 5.3 sec.

Methods). At maturity, PEA-exposed and PEA-naive fish were marked and released downstream of the divided portion of the channel to determine any baseline preferences they might have for either arm in the absence of PEA. Results indicated that, without PEA present, both PEA-exposed and PEA-naive fish tended to avoid one arm of the divided channel (arm B; Fig. 4, unshaded bars). We subsequently metered PEA into the inlet of the less preferred arm (arm B) to give an overall concentration of 10^{-7} M. When fish were tested under these conditions, PEA-naive salmon continued to select arm B at a level statistically indistinguishable from responses in the absence of PEA (Fig. 4, "PEA-naive"; compare open and shaded bars). In contrast, fish that had been exposed to PEA during parr–smolt transformation displayed a statistically significant increased tendency to select arm B once it was scented with PEA (Fig. 4; "PEA-exposed"; $\chi^2 = 10.04$; $P < 0.05$; χ^2 contingency analysis) (21).

DISCUSSION

Our results show that exposure to micromolar concentrations of PEA for as little as 10 days during a sensitive period for olfactory imprinting (parr–smolt transformation) was correlated with a specific increase in the responsiveness of olfactory receptor cells to PEA when tested 6–9 months later. Furthermore, this same exposure caused an increased behavioral preference of mature fish for micromolar concentrations of PEA when these fish would normally be returning home. These behavioral responses are compelling since

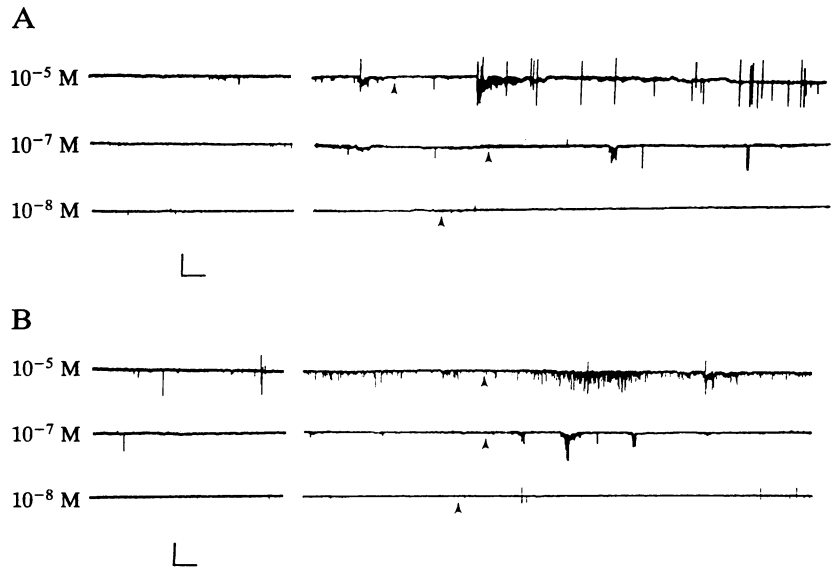

FIG. 2. L-Serine-induced activity recorded from olfactory receptor cells isolated from experimental (PEA-exposed, *A*) and control (PEA-naive, *B*) fish. Each trace was recorded from a different cell. Methods and presentation are as in Fig. 1. Calibrations: 8 pA; 4 sec.

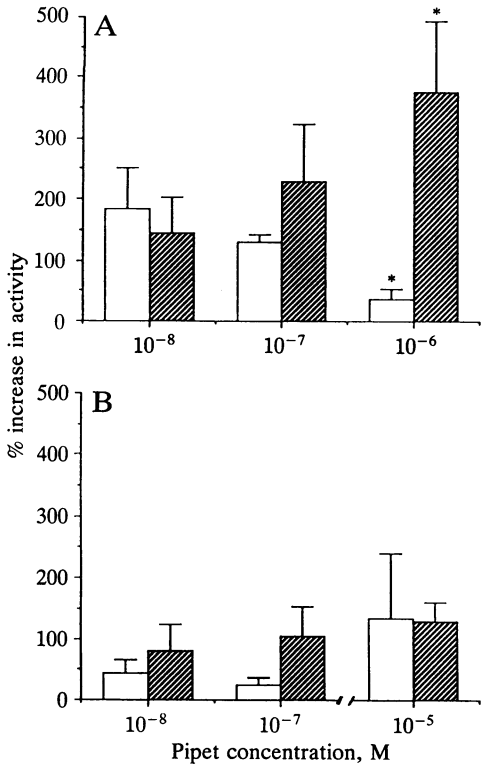

FIG. 3. Quantified responses of receptor cells to PEA and L-serine. Data plotted are average current values of odorant responses as a percentage of the average values for background activity before odorant application. Shaded bars are responses from cells isolated from PEA-exposed fish; open bars are responses from cells isolated from PEA-naive fish. All data are presented as means ± SEM. (*A*) PEA responses. The number of cells tested is as follows: 10^{-8} M, 8 naive and 8 exposed; 10^{-7}, 14 and 10; 10^{-6}, 7 and 9. The difference at 10^{-6} M is significant at $P < 0.05$ (Mann–Whitney U test). (*B*) L-Serine responses. The number of cells tested is as follows: 10^{-8} M, 7 naive and 8 exposed; 10^{-7}, 6 and 6; 10^{-5}, 8 and 6. Differences were not significant at any concentration tested. Results are based on data collected from >40 fish.

salmon reared exclusively in a hatchery may have impaired homing ability or motivation compared to wild fish (A.H.D., unpublished data). Together, these results suggest that a functional plasticity in the peripheral olfactory system may play a role in olfactory imprinting.

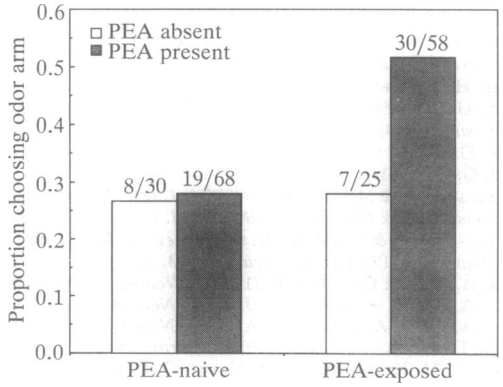

FIG. 4. Behavioral responses of PEA-exposed and PEA-naive salmon to PEA in an experimental two-choice arena. Open bars show the proportion of salmon choosing arm B in the absence of added PEA; shaded bars show the proportion of salmon choosing arm B in the presence of PEA metered into arm B. Values above bars indicate the number of fish choosing arm B/total number of fish choosing arm A or B. Differences in the responsiveness to PEA were significantly different between the two groups (see text).

To date, most studies of olfactory imprinting have focused primarily on birds and mammals. These studies have implicated changes in central processing in the main or accessory olfactory bulb in the formation of olfactory memories (27). For example, the phenomenon of "pregnancy block" in female mice is considered a model system for studying olfactory imprinting (28). During a critical period after mating, female mice form a long-term memory of urinary odor cues from the mating male. Subsequent exposure to the pheromones of an unfamiliar male initiates neuroendocrine mechanisms in the recently mated female that terminate pregnancy. If the female is exposed to the odor of the familiar male, however, the pregnancy is not terminated. Pharmacological and lesion studies indicate that memory of the mating male's pheromones is formed at the level of synaptic connections of mitral cells in the accessory olfactory bulb (28). Similarly, formation of olfactory memory in other mammals including sheep, ferrets, rabbits, and rats is thought to occur in the olfactory bulb (27, 29). Though changes in central processing are probably also important in salmonid olfactory imprinting, our results indicate that odorant-induced changes in the peripheral olfactory system may also play a role in establishing a long-term imprinted olfactory memory in vertebrates.

How might odorant exposure during a sensitive period alter receptor sensitivity? In salmon, as in mammalian systems, hormonal regulation is thought to be important in the formation of olfactory memory. Fluctuations in plasma hormone titers, including surges in thyroid hormone levels (T_4), correlate strongly with smolting (13, 14), and it has been proposed that homestream imprinting results from a permanent, hormonally mediated adjustment in olfactory sensitivity to odorants present at smolt transformation (2). Though a direct link between increased plasma T_4 levels and olfactory imprinting has been questioned (A.H.D., unpublished data), considerable evidence suggests that olfactory learning is associated with increased thyroid hormone activity (2, 30, 31). For example, coho salmon fail to home to artificial odorants experienced at early life stages when thyroid activity is comparatively low. However, artificially elevating T_4 to smolting levels has been shown to stimulate precocial imprinting, even at early life history stages (32, 33).

Since thyroid hormones are known to couple to protein synthesis via nuclear receptors, these hormones might directly alter olfactory receptor cell sensitivity to homestream odors. Evidence from catfish suggests that undifferentiated precursor cells do not yet express odorant receptors (34). Therefore, it is unlikely that a hormonally primed receptor cell sensitivity to specific imprinted odorants could occur in undifferentiated precursor cells in salmon. Instead, this finding implies that this change in receptor neuron sensitivity occurs after olfactory receptor neurons have matured to the point of expressing odorant receptors. Our data suggest two possible mechanisms of hormone action: First, binding of specific ligands (odorants) to membrane-bound receptors in the presence of hormone could trigger an increased functional expression of those receptor proteins (35–40). In an alternative scenario, hormones could promote a proliferation of olfactory receptor neurons that are sensitive to a wide variety of odors present in the homestream environment. Different clones of receptor neurons that were most active (i.e., responsive to homestream odorants) might then survive to find synaptic targets in the olfactory bulb, thus establishing a peripheral homestream memory. This punctuated proliferation and selective survival of olfactory receptor neurons could speculatively lead to a reorganization of glomerular structures within the bulb as well.

In support of the proliferation/clonal selection hypothesis, results from recent studies challenge established dogma that olfactory receptor cells undergo continuous replacement or

"turnover" throughout life (41) and implicate thyroid hormones as regulators of neurogenesis and maintenance in the peripheral olfactory system. For example, thyroid hormones (T_3 and T_4) promote neurogenesis and cyto-architectural changes in peripheral olfactory systems of other vertebrates, while hypothyroidism reduces turnover of populations of olfactory receptor neurons (42–46). In addition, thyroid hormones are also well established as selective promoters of cyto-architectural changes, including increases in dendritic arborization of neurons (47–50), synaptogenesis in the central nervous system (51), and increased functional expression of specific membrane receptors (38, 39, 52, 53).

Recent work with salmon further suggests that the peripheral olfactory system is plastic and may be sensitive to hormonal events, particularly at parr–smolt transformation. For example, patch clamp studies of coho salmon (*Oncorhynchus kisutch*) have documented consistent differences in outward current components in olfactory receptor cells isolated before and after smolting, possibly reflecting differences in the receptor cell population at these two life stages (17). Anatomical studies of Atlantic salmon (*Salmo salar*) suggest a quadrupling of olfactory receptor cell number during smolting as well as specific modifications in the olfactory bulb neuropil during this transition (54). Although the potential involvement of thyroid hormones in promoting these neuroanatomical changes is not known, recent studies indicate an enrichment of T_3 receptors in the olfactory epithelium of smolting masu salmon (*Oncorhynchus masou*).¶ Finally, studies of rainbow trout (*Oncorhynchus mykiss*) show nine distinct terminal olfactory receptor cell projection fields ranging in size from 1% to 35% of the glomerular layer, but the functional relevance of this segregation is unclear (55, 56). Questions concerning the natural development as well as the sensitivity of these projection fields to hormonal modulation are waiting to be addressed.

In conclusion, our results show that isolated ciliated olfactory receptor cells are electrically responsive to behaviorally appropriate concentrations of known odorants. Our results further suggest a behaviorally relevant difference in the responsiveness of receptor neurons to PEA, depending on prior exposure of fish to this odorant during parr–smolt transformation. This result does not contradict evidence implicating forebrain plasticity in establishing imprinting but implies that some component of olfactory imprinting may be occurring in the periphery.

¶Yamauchi, K., Kudo, H., Shimuzu, M., Tsuneyoshi, Y. & Ueda, H., International Conference and Workshop on Homing and Straying in Salmon, Oct. 25–29, 1993, Roros, Norway, abstr. 27.

We dedicate this work to the memory of our friend and colleague, John Konecki. We are indebted to E. Brannon, S. Courtney, J. Konecki, J. Meyers, T. The Vu, G. Yokoyama, and especially B. Smith and J. Silverstein for advice and assistance in rearing fish. Computer and statistical analyses were much improved by D. Pentcheff and T. Wootton. The comments of A. Bass, M. Bosma, R. Brantley, W. Dickhoff, D. Lenzi, C. Miles, L. Riddeford, W. Roberts, D. Willows, and three anonymous reviewers greatly improved the presentation of this manuscript. This work was supported in part by National Institutes of Health Training Grants to G.A.N. and A.H.D. (GM07108-14 and National Institute on Environmental Health Science ES07032), a Sigma XI grants-in-aid-of-research award to G.A.N., the Graduate School Research Fund of the University of Washington, and hatchery facilities at the University of Washington School of Fisheries and Seward Park. Additional support was provided by the National Science Foundation (Grant BNS8908697 to T.P.Q.), the National Institutes of Health (Grant HD17486 to W.J.M.), and a Research Career Development Award to W.J.M.

1. Quinn, T. P. & Dittman, A. H. (1990) *Trends Ecol. Evol.* **5**, 174–177.
2. Hasler, A. D. & Scholz, A. T. (1983) *Olfactory Imprinting and Homing in Salmon* (Springer, New York).
3. Scholz, A. T., Gross, C. K., Cooper, J. C., Horrall, R. M., Hasler, A. D., Daly, R. I. & Poff, R. J. (1978) *Trans. Am. Fish. Soc.* **107**, 439–443.
4. Scholz, A. T., Horrall, R. M., Cooper, J. C. & Hasler, A. D. (1976) *Science* **192**, 1247–1249.
5. Sutterlin, A. M. & Gray, R. (1973) *J. Fish. Res. Board. Can.* **30**, 985–989.
6. Cooper, J. C. & Hasler, A. D. (1974) *Science* **183**, 336–338.
7. Cooper, J. C. & Hasler, A. D. (1976) *J. Fish. Res. Board. Can.* **33**, 688–694.
8. Dodson, J. J. & Bitterman, M. E. (1989) *Biol. Behav.* **14**, 13–27.
9. Hara, T. J. & Brown, S. B. (1979) *J. Fish. Res. Board Can.* **36**, 1186–1190.
10. Hara, T. J., MacDonald, S., Evans, R. E., Murui, T. & Arai, S. (1984) in *Mechanisms of Migration in Fishes*, eds. McCleave, J. D., Arnold, G. P., Dodson, J. D. & Neill, W. H. (Plenum, New York), pp. 363–378.
11. Nevitt, G. A. & Moody, W. J. (1989) *Soc. Neurosci. Abstr.* **15**, 748.
12. Nevitt, G. A. (1990) Ph.D. thesis (Univ. of Washington, Seattle).
13. Hoar, W. S. (1988) in *Fish Physiology*, eds. Hoar, W. S. & Randall, D. J. (Academic, New York), Vol. 11, pp. 275–343.
14. Folmar, L. C. & Dickhoff, W. W. (1980) *Aquaculture* **21**, 1–37.
15. Clarke, W. C. & Blackburn, J. (1977) *Dept. Environ. Can. Fish. Mar. Serv. Res. Div. Dir. Tech. Rep.* **705**, 1–11.
16. Sullivan, C. V. (1988) Ph.D. thesis (Univ. of Washington, Seattle).
17. Nevitt, G. A. & Moody, W. J. (1992) *J. Exp. Biol.* **166**, 1–17.
18. Crank, J. (1990) *The Mathematics of Diffusion* (Clarendon, Oxford), p. 29.
19. Maue, R. A. & Dionne, V. E. (1987) *J. Gen. Physiol.* **90**, 95–125.
20. Dionne, V. E. (1992) *J. Gen. Physiol.* **99**, 415–433.
21. Zar, J. H. (1984) *Biostatistical Analysis* (Prentice-Hall, Englewood Cliffs, NJ), 2nd Ed.
22. Brannon, E. L., Feldmann, C. & Donaldson, L. (1982) *Aquaculture* **28**, 195–200.
23. Rehnberg, B. G., Jonasson, B. & Schreck, C. B. (1985) *Trans. Am. Fish. Soc.* **114**, 732–736.
24. Idler, D. R., Fagerlund, U. H. & Mayoh, H. (1956) *J. Gen. Physiol.* **39**, 889–892.
25. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981) *Pflügers Arch.* **391**, 85–100.
26. Kostyuk, P. G. (1980) *J. Neurosci.* **5**, 945–959.
27. Hudson, R. (1993) *Curr. Opinion Neurobiol.* **3**, 548–552.
28. Brennan, P., Kaba, H. & Keverne, E. B. (1990) *Science* **250**, 1223–1226.
29. Leon, M. (1992) *Trends* **10**, 34–38.
30. Morin, P. P., Dodson, J. J. & Dore, F. Y. (1989) *Can. J. Fish. Aquat. Sci.* **46**, 122–130.
31. Morin, P. P., Dodson, J. J. & Dore, F. Y. (1989) *Can. J. Fish. Aquat. Sci.* **46**, 131–136.
32. Scholz, A. T. (1980) Ph.D. thesis (Univ. of Wisconsin, Madison).
33. Scholz, A. T., White, R. J., Muzi, M. & Smith, T. (1985) *Aquaculture* **45**, 199–214.
34. Ngai, J., Chess, A., Dowling, M. M., Necles, N., Macagno, E. R. & Axel, R. (1993) *Cell* **72**, 667–680.
35. Lancet, D. (1986) *Annu. Rev. Neurosci.* **9**, 329–355.
36. Hill, D. L. & Przekop, P. R. (1988) *Science* **241**, 1826–1828.
37. Buck, L. & Axel, R. (1991) *Cell* **65**, 175–187.
38. Oppenheimer, J. H., Schwartz, H. L., Mariash, C. N., Kinlaw, W. B., Wong, N. C. W. & Freake, H. C. (1987) *Endocr. Rev.* **8**, 288–308.
39. Sammuels, H. H., Forman, B. M., Horowitz, Z. D. & Ye, Z. S. (1989) *Annu. Rev. Physiol.* **51**, 623–639.
40. Wang, H., Wysocki, C. J. & Gold, J. H. (1993) *Science* **260**, 998–1000.
41. Burd, G. D. (1990) in *The Proceedings of the Tenth International Symposium on Olfaction and Taste*, ed. Døving, K. B. (Univ. of Oslo Press, Oslo), pp. 196–205.
42. Burd, G. D. (1991) *J. Comp. Neurol.* **304**, 123–134.
43. Paternostro, M. & Meisami, E. (1989) *Int. J. Dev. Neurosci.* **7**, 243–255.
44. Paternostro, M. & Meisami, E. (1991) *Int. J. Dev. Neurosci.* **9**, 439–452.
45. Mackay-Sim, A. & Beard, M. D. (1987) *Dev. Brain Res.* **36**, 190–198.
46. Farbman, A. I. (1990) *Trends NeuroSci.* **13**, 362–365.
47. Hoskins, S. G. & Grobstein, P. (1985) *J. Neurosci.* **5**, 930–940.
48. Rami, A., Rabie, A. & Patel, A. J. (1986) *Neuroscience* **19**, 1207–1216.
49. Rami, A., Patel, A. J. & Rabie, A. (1986) *Neuroscience* **19**, 1217–1226.
50. Gould, F. & Butcher, L. L. (1989) *J. Neurosci.* **9**, 3347–3358.
51. Hofmann, M. H., Michler, A. & Meyer, D. L. (1989) *Brain Res.* **492**, 389–491.
52. Glass, C. K. & Holloway, J. M. (1990) *Biochim. Biophys. Acta* **1032**, 157–176.
53. Brent, A. G., Moore, D. D. & Larsen, P. R. (1991) *Annu. Rev. Physiol.* **53**, 17–35.
54. Bowers, S. M. (1988) Thesis (Yale Univ., New Haven, CT).
55. Riddle, D. R. & Oakley, B. (1991) *J. Neurosci.* **11**, 3752–3762.
56. Riddle, D. R. & Oakley, B. (1992) *J. Comp. Neurol.* **324**, 575–589.